REMARKS

35 U.S.C. § 101

Applicants submitted a complete response to the Office action mailed September 20, 2005 on November 21, 2005. In that response, Applicants submitted declaratory evidence in the form of a Declaration by Paul Polakis, Ph.D., establishing that, in general, the correlation between gene amplification and protein overexpression is art-accepted. However, the Advisory action mailed January 24, 2006 maintains rejection of Claims 27-34 for alleged lack of utility. In maintaining rejection of Claims 27-34, the Advisory action states that the Polakis Declaration is not persuasive because (1) the declaration "does not provide data such that the examiner can independently draw conclusions;" (2) allegedly "there is no evidentiary support to Dr. Polakis' statement that it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded polypeptide;" and (3) the literature, *e.g.* Hu *et al.,* "cautions researchers from drawing conclusions based on small changes in transcript expression levels between normal and cancerous tissue."

Applicants respectfully disagree with the above-stated bases for finding that the Polakis Declaration is not persuasive evidence establishing that the claimed polypeptides are supported by a substantial utility. Specifically, Applicants disagree that there is no evidentiary support for Dr. Polakis's statement and Applicants disagree that the literature generally cautions against drawing conclusions based on changes in transcript expression levels. Indeed, numerous art references, including the Orntoft, Pollack, Varis, Bermont, and Hu references previously submitted and the Papotti, Walmer, Janssens, Hahnel, Kammori, Maruyama, Bea, and Futcher references submitted herewith establish that generally gene amplification correlates with protein overexpression. These references also illustrate that gene amplification in cancerous tissue is an art-accepted indicator of protein overexpression. Finally, although Applicants disagree that explicit data is needed to support the Polakis Declaration, Applicants herein submit a second declaration of Dr. Polakis, along with data establishing correlation between gene amplification and protein overexpression.

Compliance with 35 U.S.C. § 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) *cert. denied*, 469 US 835 (1984). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the totality of the evidence under consideration. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Thus, to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Office must establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. Indeed, an Applicant's assertion of utility is sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380,1391, 183 USPQ 288, 297 (CCPA 1974). *See, also In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (1965); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977).

Significantly, statistical certainty regarding an Applicants' assertion of utility is not required to satisfy 35 U.S.C. § 101. *Nelson v. Bowler*, 626 F.2d 853, 856-857, 205 USPQ 881, 883-884 (CCPA 1980). Where an Applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed as "wrong" even where there may be some reason to question the assertion. MPEP § 2107.02. Rather, a 35 U.S.C. § 101 rejection should only be sustained where the asserted utility *violates a scientific principle or is wholly inconsistent* with contemporary knowledge in the art. *In re Gazave*, 379 F.2d 973, 978, 154 U.S.P.Q. 92, 96 (CCPA 1967) (emphasis added).

Applicants respectfully submit that the Office fails to overcome the presumption of truth that must be applied to Applicants' assertion of utility, *see* pages 119 and 137 of the specification, because the Office fails to establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of Applicants' statement of utility. The Office also fails to establish that Applicants' assertion of utility violates any scientific principle or that it is <u>*wholly*</u> inconsistent with contemporary knowledge in the art. Indeed, it is particularly clear that the Office fails to overcome the presumption of truth

that must be applied to Applicants' assertion of utility when that assertion is considered, *as it must be,* along with the substantial evidence Applicants have cited in support of the asserted utility. This evidence clearly establishes that those of skill in the art generally accept that gene expression levels correlate to protein expression levels. Indeed, paragraphs 4-6 of the Declaration of Paul Polakis, Ph.D., submitted with the Response and Request for Reconsideration mailed November 21, 2005, illustrate the art acceptance of a correlation between mRNA levels and polypeptide levels. Significantly, Dr. Polakis declares that "in approximately 80%" of the cases observed in connection with the Tumor Antigen Project, increases in the mRNA levels correlated with changes in the levels of protein expression."

Although Applicants disagree with the allegation in the Advisory action that Dr. Polakis's declaration is not persuasive because it did not include any data that would allow the examiner to independently evaluate the data, Applicants herein submit a second declaration of Dr. Polakis, (*see* Exhibit 1, Second Declaration of Paul Polakis, Ph.D.). This second Polakis Declaration presents data demonstrating that more than 90% of the genes identified as being amplified in the Tumor Antigen Project referenced in the Polakis Declarations and in the Gene Amplification Experiment described in Example 28 of the specification, were detectably overexpressed in human tissue compared to normal tissue at both the mRNA and protein levels. *See* Paragraph 5 and Exhibit B of the Second Polakis Declaration. More specifically, in his second declaration, Dr. Polakis declares that the data provided therein indicates that "of the 31 genes identified as being detectably overexpressed in human tumor tissue as compared to normal tissue at the mRNA level, 28 of them (i.e., greater than 90%) are also detectably overexpressed in human tumor tissue as compared to normal human tissue at the protein level. As such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." See Paragraph 5 and Exhibit B of the Second Declaration of Paul Polakis, Ph.D. (emphasis original). Thus, this declaratory evidence and data clearly establish that for the claimed polypeptide, one of ordinary skill in

the art would find it more likely than not that amplification of the PRO357 nucleic acid correlates with overexpression of the PRO357 polypeptide.

Furthermore, the declaratory evidence presented in the Polakis declarations, including Dr. Polakis's statement that "it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein," is clearly consistent with knowledge in the art. Indeed, the art references discussed below establish that it is more likely than not that one of ordinary skill in the art would accept that generally, there is a correlation between gene amplification and protein overexpression.

For example, Pollack *et al.* profiled DNA copy number alterations across 6,691 mapped human genes in 44 breast tumors and 10 breast cancer cell lines and reported that microarray measurements of mRNA levels revealed remarkable degrees to which variation in gene copy number contributes to variation in gene expression in tumor cells. *See* Pollack *et al.,* "Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors." 2002. *PNAS*, 99(20):12963-12968 (submitted previously). Pollack *et al* further report that their findings that DNA copy number plays a role in gene expression levels are generalizable. Thus significantly, "[t]hese findings provide evidence that widespread DNA copy number alteration can lead directly to global deregulation of gene expression, which may contribute to the development or progression of cancer."

In particular, Pollack *et al.* report a parallel analysis of DNA copy number and mRNA levels. Pollack *et al.* found that "[t]he overall patterns of gene amplification and elevated gene expression are quite concordant, i.e., a significant fraction of highly amplified genes appear to be correspondingly highly expressed." (emphasis added). Specifically, of 117 high-level DNA amplifications 62% were associated with at least moderately elevated mRNA levels and 42% were found associated with comparably highly elevated mRNA levels.

Orntoft *et al.* report similar findings in "Genome-wide study of gene copy numbers, transcripts, and protein levels in pairs of non-invasive and invasive human transitional cell carcinomas." 2002. *Molecular & Cellular Proteomics* 1.1, 37-45 (submitted

previously). Initially, Orntoft *et al.* note that "[h]igh throughput array studies of the breast cancer cell line BT474 ha(ve) suggested that there is a correlation between DNA copy numbers and gene expression in highly amplified areas ( ), and studies of individual genes in solid tumors have revealed a good correlation between gene dose and mRNA or protein levels in the case of c-erb-B2, cyclin d1, ems1, and N-myc."

Specifically, Orntoft *et al.* used 2D-PAGE analysis on four breast tumor tissue samples to determine correlation between genomic and protein expression levels of 40 well resolved, known proteins. Orntoft reported that "[i[n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations ( ). Only one gene showed disagreement between transcript alteration and protein alteration." (emphasis added). Additionally, Orntoft *et al.* report that "11 chromosomal regions where CGH showed aberrations that corresponded to the changes in transcript levels also showed corresponding changes in the protein level ( )." The regions examined by Orntoft include genes encoding proteins that are often found altered in bladder cancer.

Orntoft *et al.* note that their study reports a striking correspondence between DNA copy number, mRNA expression and protein expression. Orntoft *et al.,* further note that any observed discrepancies in correlation may be attributed to translation regulation, post-translation processing, protein degradation or some combination of these. *See also* Hyman *et al.,* "Impact of DNA amplification on gene expression patterns in breast cancer." 2002. *Cancer Research*, 62:62-40-6245 (submitted previously).

Varis, Bermont, Papotti, Walmer, Janssens, Hahnel, Kammori, Maruyama, and Bea are yet further examples that utility of the present invention, which is based on a correlation between gene amplification and protein overexpression, is not wholly inconsistent with knowledge in the art. Varis et al., carried out a comprehensive analysis of gene copy number and expression levels of 636 chromosome 17-specific genes in gastric cancer. *See* Varis et al., "Targets of gene amplification and overexpression at 17q in gastric cancer." *Cancer Res.* 2002. 1;62(9):2625-9 (submitted previously). Specifically, Varis *et al.* report that analysis of DNA copy number changes by comparative genomic hybridization on a cDNA microarray revealed increased copy numbers of 11 genes, 8 of

which were found to be overexpressed in the expression analysis. Thus, Varis *et al.*, teach there is a 72% correlation between increased DNA copy number and gene expression level.

Bermont teaches that overexpression of p185 is usually associated with c-erbB-2 amplification. Specifically, Bermont reports that 100% of the overexpressed p185 protein in 106 breast cancer samples studied also displayed c-erbB-2 amplification. *See* Bermont *et al.*, "Relevance of p185 HER-2/neu oncoprotein quantification in human primary breast carcinoma." *Breast Cancer Res Treat.* 2000 63(2):163-9 (submitted previously). *See also* Hu *et al.*, "Profiling of differentially expressed cancer-related genes in esophageal squamous cell carcinoma (ESCC) using human cancer cDNA arrays: overexpression of oncogene MET correlates with tumor differentiation in ESCC." *Clin Cancer Res.* 2001 7(11):3519-25 (the results of cDNA arrays showed that 13 cancer-related genes were upregulated > or = 2 fold and immunostaining results of the expression of the MET gene showed MET overexpression at the protein level, validating the cDNA arrays findings) (submitted previously).

Papotti *et al.* (*Diagn Mol Pathol.* 9(1):47-57 (2000); (submitted herewith) studied the somatostatin type 2 receptor (sst2) in 26 different neuroendocrine lung tumors. They investigated mRNA levels by RT-PCR and protein levels by immunohistochemistry using 2 different antibodies. The authors report that "in the majority of samples a good correlation between sst2 mRNA (as detected by RT-PCR) and sst2 protein expression (as detected by immunohistochemistry) was observed" (Abstract). The authors also performed in situ hybridization (ISH) in selected samples which "paralleled the results obtained with the other techniques" (Abstract).

Walmer *et al.* (*Cancer Res.* 55(5):1168-75 (1995); submitted herewith) looked at lactoferrin mRNA and protein expression in endometrial adenocarcinomas and report that two thirds (8 of 12) of the samples examined overexpress lactoferrin. Walmer *et al.* also found that "this tumor-associated increase in lactoferrin expression includes an elevation in the mRNA and protein of individual cells" and that "serial sections of malignant specimens show(ed) a good correlation between the localization of lactoferrin mRNA and protein in

individual epithelial cells by in situ RNA hybridization and immunohistochemistry" (Abstract).

Janssens *et al.* (*Tumour Biol.* 25(4):161-71 (2004); submitted herewith) evaluated the involvement of frizzled receptors (Fzds) in oncogenesis. They investigated mRNA expression levels in 30 different human tumor samples and their corresponding (matched) normal tissue samples by real-time quantitative PCR. Janssens *et al.* observed markedly increased Fzd5 mRNA levels in 8 of 11 renal carcinoma samples and that "Western blot analysis of crude membrane fractions revealed that Fzd5 protein expression in the matched tumor/ normal kidney samples correlated with the observed mRNA level" (Abstract).

Hahnel *et al.* (*Breast Cancer Res Treat.* 24(1):71-4 (1992); submitted herewith) studied expression of the pS2 gene in breast tissues by measuring mRNA levels using Northern blotting and protein levels by radioimmunoassay. Hahnel *et al.* indicate that "there was a good correlation between the two measurements, indicating that expression of the pS2 gene in breast tissues may be assessed by either method."

Kammori *et al.* (*Int J Oncol.* 27:1257-63 (2005); submitted herewith) studied the expression of human telomerase reverse transcriptase (hTERT) gene and protein (besides estrogen and progesterone receptors) in breast tumors using in situ hybridization (ISH) for mRNA and immunohistochemistry (IHC) for the protein. They looked at 64 adenocarcinomas, 2 phyllode tumors and their adjacent normal breast tissues and found that hTERT mRNA was detected in 56 tumors but in neither of the 2 phyllode tumors whereas hTERT protein expression was detected by IHC in 52 tumors but in neither of the 2 phyllode tumors. The authors concluded that "there was a strong correlation between detection of hTERT gene expression by ISH and of hTERT protein by ICH in tissue specimens from breast tumors" (Abstract).

Maruyama *et al.* (*Am. J. Pathol.* 155:815-822 (1999); submitted herewith) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that all three Id mRNA species

were expressed at high levels in pancreatic cancer cells as compared to normal or CP samples, and that the pancreatic cancer cell lines also exhibited "a good correlation between Id mRNA and protein levels" (Abstract). The authors measured both mRNA and protein expression in five different human pancreatic cancer cell lines. The authors observed a correlation between mRNA and protein expression of Id1 in all five cell lines, and a correlation between mRNA and protein expression for Id2 and Id3 in four out of five cell lines. In these discordant cases, Id protein levels were increased while mRNA levels were not. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility. Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Bea *et al.* (*Cancer Res.* 61:2409-2412 (2001); submitted herewith) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in lymphoma samples. The authors examined BMI-1 protein expression in 31 tumors for which levels of gene amplification and mRNA expression had been determined. Bea *et al.* found that "[a] good correlation between BMI-1 mRNA levels and protein expression was observed in all types of lymphomas" (Abstract). Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Applicants also submit an additional reference in support of the assertion that in general, mRNA expression levels are correlated with protein expression levels. Futcher *et al.* (*Mol. Cell. Biol.* 19:7357-7368 (1999) (submitted herewith)) analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.*, cited in the Office action and discussed more fully in the response submitted November 21, 2005, completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* note that this is in part a difference in viewpoint, in that "Gygi *et al.* focus on the fact that the correlations of mRNA and codon bias with protein abundance are far from perfect" (page 7367, col. 1). Applicants respectfully submit that a showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predicts protein overexpression. The data of both Futcher *et al.* and Gygi *et al.* clearly meets this standard.

Futcher *et al.* also point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* first note that Gygi *et al.* used the Pearson product-moment correlation coefficient (rp) to measure the covariance of mRNA and protein abundance. Futcher *et al.* point out that "the rp correlation is a parametric statistic and so requires variates following a bivariate normal distribution; that is, it would be valid only if both mRNA and protein abundances were normally distributed" (page 7367, col. 1; emphasis added). As the authors disclose, "both distributions are very far from normal," and thus "a calculation of rp is inappropriate" (page 7367, col. 1).

In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (rs), an nonparametric statistic that does not require the data to be normally distributed. Using the rs, the authors found that mRNA abundance was well correlated with protein abundance (rs = 0.74). Applying this statistical approach to the data of Gygi *et al.* also resulted in a good correlation (rs = 0.59), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an rp. Two types of transformation (Box-Cox and logarithmic)

were used, and both resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots. In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation in vivo" (page 7367, col. 2).

Accordingly, the results of Futcher *et al.* demonstrate "a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2). Further, Futcher *et al.* show that when corrected for an inappropriate statistical analysis and systematic error in the measurement of low abundance proteins, the data of Gygi *et al.* also meets the "more likely than not standard" and shows that a positive correlation exists between mRNA levels and protein levels.

Thus, although there may not always be a 100% correlation between gene amplification and protein overexpression, the above-discussed references and the Polakis declarations evidence that the utility of the present invention does not violate any scientific principle, nor is it wholly inconsistent with the knowledge in the art. Indeed, these references and the declarations evidence that one of ordinary skill in the art would find it more likely than not

that gene amplification of PRO357 correlates with overexpression of the PRO357 polypeptide and would accept the asserted diagnostic utility of the claimed antibodies as a specific, substantial, and credible utility.

The Advisory action also cites Hu *et al.* as evidence that the art cautions against drawing conclusions based on small changes in transcript levels. However, the conclusions of Hu *et al.* are based upon statistical analysis of information obtained from published literature, and not from experimental data. Nowhere does Hu *et al.* discuss any information on microarray experiments, for example, the control used in the assays. In addition, Hu *et al.* assessed the biological significance of genes identified by microarray assay solely based on the frequency of literature citations of these genes, which does not reflect the true biological significance of these genes. Therefore, the statistical analyses by Hu *et al.,* is neither reliable nor informative.

Applicants respectfully submit that the Office and Advisory actions fail to set forth a prima facie case of lack of utility. However, even if the Office maintains that a prima facie case of lack of utility is established, consideration of the totality of the evidence, including the evidence presented by Applicants as discussed above, and the references cited by the Office, both in the previous Office actions and in the Advisory action, clearly demonstrates that the one of ordinary skill in the art would not find it more likely than not that, in general, there is no correlation between gene amplification and protein overexpression. Indeed, the totality of the evidence shows that the proposition that there will be correlation between protein and transcript levels does not violate any scientific principles nor is it wholly inconsistent with knowledge in the art.

Thus, although Applicants respectfully disagree with the Office's rejection of Applicants' additional asserted utility based on the Ashkenazi Declaration (submitted previously along with the teachings of Hanna and Mornin, which support the Ashkenazi Declaration *see* (Pathology Associates Medical Laboratories, August (1999)), Applicants need not rely on this additional asserted utility. The law only requires that an applicant provide "one credible assertion of a specific and substantial utility for each claimed invention to satisfy the utility requirement." See MPEP § 2107 (emphasis added). As explained above,

Applicants have clearly established that it is more likely than not that one of ordinary skill in the art would accept that generally gene amplification correlates with protein overexpression and based on this correlation one of ordinary skill in the art would find that Applicants' assertion of utility does not violate, nor is it inconsistent with any scientific principles. Therefore, Applicants have overcome rejection of claims 27-34 for alleged lack of utility for the reasons discussed above and respectfully request that this ground of rejection be withdrawn.

35 U.S.C. § 112 ¶ 1, Enablement-Utility

Claims 27-34 stand rejected under 35 U.S.C. § 112 ¶1, because it is alleged that the presently claimed invention is not supported by a substantial utility, and therefore, one skilled in the art would not know how to use the claimed invention. As discussed in the remarks above, addressing the rejection under 35 U.S.C. § 101 for lack of utility, Applicants respectfully submit that the claimed polypeptide is supported by a substantial utility. Thus, Applicants respectfully request the Examiner reconsider and withdraw this ground of rejection.

SUMMARY

Applicants believe that currently pending Claims 27-34 are patentable. The Examiner is invited to contact the undersigned attorney for Applicants via telephone if such communication would expedite allowance of this application.

Respectfully submitted,

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